# EFFECTS OF VITAMINS

# ON

# THYROID AND SUPRARENALS.

( By R. K. PAL.- Department of Physiology.

UNIVERSITY OF EDINBURGH.

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## Part I.

## Effects of vitamins A and B on thyroid and suprarenals.

There are no two opinions about the fact that vitamins are the most important of all the accessory food factors. This paper deals with the structural changes in the thyroid and suprarenal glands as affected by the presence or absence of vitamin A or B in the diet.

The experiments were mainly performed on rats and mice in batches , kept on diets deficient in vitamins A and B respectively, while others which served as controls were getting fresh butter and powdered wheat-germs as an addition of vitamins A and B respectively. The Vitamin A deficient animals were getting an equal quantity of margarine so that the fat content of their diet was equal to that of the controls. The basal diet of both the batches contained, Indian corn, wheat gluten, Calcium carbonate, sodium chloride and skimmed milk, so that even the diet of the first group of animals apart from deficiency of vitamin A was otherwise an adequate one. The basal diet of the vitamin B-deficient animals as well as their controls was an adequate quantity of the mixture of meat-extract, white flour,calcium carbonate, common salt and

and milk.This diet too so far as factors other than vitamin B were concerned was surely an adequate one.

Eight animals on a vitamin A deficient diet showed an average loss of 28.25 gms, while eight animals used as controls gained 39.35 gms after feeding for eight weeks. Four of the deficiency stock developed eye symptoms of xerophthalmia.

Eight animals kept on a diet deficient in vitamin B for eight weeks showed an average loss of 12.875 gms, whereas the controls gained 12.75 gms. Two of the former group deeveloped symptoms of polyneuritis,such as inability to walk, weakness and general asthenia.

The metabolism of these experimental animals was recorded twice every week. Animals taking vitamin A exhibited a slowly progressive diminution in metabolism as indicated by the oxygen consumed,so that at the end of the experimental period, the batches of four consumed about 160 c.c. oxygen less than formerly; whereas those on diet deficient in vitamin A had an almost unaltered metabolism showing a difference of 35 c.c. only, inoxygen cohsumption than formerly. Those on a diet deficient in vitamin B showed an increase of oxygen consumption of 95 c.c. per hour ; animals on vitamin B containing diet exhibited a slight diminution of metabolism ( a fall of 45 c.c. of oxygen consumption per hour ).

The thyroid gland of animals deficient in vitamin A showed an average gain of 5 mgms, and the suprarenal glands an excess of 3.25 mgms. over the average controls. The thyroid of vitamin B deficient animals had on the

on the average a gain of 4.9 mgms, and the suprarenal a loss of 2.5 mgs compared with the controls. The size and weight of the thyroid of the female animals exhibited much more change than that of the males.

Histologically, the thyroid of vitamin A deficient animals was found to be of actively secreting type, the vesicles containing very little colloid material; whereas the glands of the controls showed a resting condition of the glands with thin secreting epithelium and vesicles full of colloid material exactly as in the animals fed with thyroid.(1).The glands of two rats that received one grain of thyroid extract each daily, for a fortnight showed exactly similar structure. McCarrison (2) noticed a similar effect of diet containing butter and oniom in the thyroid glands of monkeys.The glands of vitamin B deficient animals showed signs of active secretion similar to that of vitamin A deficient animals but to a greater degree as indicated by hyperplasia and fibrosis of the glands and the secreting epithelium proliferating into the vesicles and almost obliterating them. The glands of those animals that developed polyneuritis specially exhibited these changes.

Staining with neutral red by the intravitam method showed a scanty number of phagocytic cells in the vitamin A deficient glands in comparison with the controls. This was not present in the vitamin B deficient glands.

THE ACETONITRIL TEST:-

Following Reid Hunt's suggestion(3) that activity of thyroid gland can be estimated by injection of aceto-nitril in mice by noting their resistance to this drug, I tried the same test in vitamin A fed and vitamin A deficient mice. The minimum fatal dose of the drug in terms of body weight was worked out by Miura.(4). Following this, my injection began with half a minim dose. Of the four animals kept on deficiency diets for six weeks, three died of the first injection while one died with 3/4 m.dose: whereas three of the four animals on vitamin A containing diet, all resisted up to 1 m. dose except one which died with a 3/4 m. dose.But when animals on deficient diets were given thyroid extract,half grain per day for seven days, they could resist much larger doses of acetonitril e.g $3\frac{1}{2}$ m.The injection of the same drug in animals on vitamin B containing diets, failed to show any increased resistance of the animals to the drug over the deficient group, almost all of the six animals of both the batches dying of injection of a dose of 3/4m. each.

The loss in weight of the animals on vitamin A deficient diet, the gain in the weight of the thyroid glands, the increased oxygen consumption and histological sections stained by different methods, all show that vitamin A deficiency leads to a definite-ly increased activity of the thyroid glands. That other

other factors such as cold also lead/ to an extreme degree of activity of the gland, has been shown by Cramer.(5). Due to this continual secretory activity, the gland cells become exhausted, and however much they may try to meet an emergency such as the acetonitril poisoning,they ultimately fail and death results. Histological sections of the glands of animals, whether on deficiency or vitamin containimg diet dying as a result of acetonitril injection, all showing signs of active secretion, corroborate this statement.

On the other hand the control animals on diet containing vitamin A, exhibit a resting condition of the thyroid glands as indicated by their gain in weight,diminution in size and diminished weight of thyroid glands and microscopical sections stained by various methods. The glands are evidently in a resting phase, ready to exert their detoxicating function by increased secretion; that is perhaps why these animals are able to resist about double the dose of acetonitril, that is fatal for animals fed on deficiency diet.In this respect vitamin A seems to have an influence on the thyroid gland such as thyroid feeding itself possesses. Animals fed first on deficiency diet and then on thyroid for a week show changes in the gland similar to that in vitamin-fed animals, where all the actively secreting gland cells become converted into resting ones,the vesicles being full of colloid material. In the hands of Reid Hunt thyroid feeding led to an increased resistance of mice to acetonitril injection which

which was no doubt due to the power of the glands to meet the emergency to an extent by assuming an active role after their activity was held in abeyance by thyroid feeding. The fact that the glands of animals on thyroid, that have died after an increased dose of acetonitril, show signs of activity, proves clearly that the glands tried their utmost to secrete as much as they could before succumbing to the toxic effect of the drug. In this respect increased resistance of the resting glands to acetonitril may be partly due to the increased number of phagocytic cells, found by intravitam method of staining with neutral red. Failure of vitamin B to exert a detoxicating function in the body though partly behaving similarly to vitaminA in promoting rest to the gland, may be explained by the peculiar failure of response of the phagocytic cells on administration of vitamin B.

## SUPRARENAL GLAND.

Cramer (6) by his osmic acid vapour method of staining showed how complete avitaminosis leads to a total absence of lipoid material from the suprarenal cortex. By the same method, glands of animals on diets lacking in vitamin A showed a slight diminution in the lipoid content of the cortex. The medullary cells showed signs of active secretion and contained a greater quantity of adrenaline granules, than in the glands of the controls. The glands of the animals on vitamin B deficient diet exhibited much more diminution of the lipoid content in their cortex but less quantity of adrenaline granules in the medulla as compared with the

controls.The cells of the medulla of the glands of both the batches appeared to be in a moderately active phase.

Quantity of adrenaline in suprarenal glands of the vitaminA-fed animals by Folin, Cannon and Denis Method.(7).

| No | Weight of animal | Wt of gland | Total Adrenaline | Percentage of adrenaline |
|---|---|---|---|---|
| 10 | 170 gms. | 68mgms | 0.028764mgms | 0.0432% |
| 3 | 230 " | 65" " | 0.028860 " | 0.0444% |
| 11. | 185 " | 51 " | 0.021216 " | 0.0416% |
| 2. | 340 " | 57 " | 0.027132 " | 0.0476% |
| Average | 231.25 gms | 60.25mgms | 0.026493 mgms | 0.043975% |

Quantity of adrenaline in the suprarenal glands of the vitamin A deficient Animals.

| No | Wt of animals | Wt of gland | Total adrenaline | percentage |
|---|---|---|---|---|
| 14. | 150 gms | 55 mgms | 0.027115 mgms | 0.0493 % |
| 16 | 225 " | 50 " | 0.024200 " | 0.0484% |
| 15 | 190 " | 92 " | 0.07636 " | 0.0833% |
| 7 | 225 " | 60 " | 0.039308 " | 0.0 634% |
| Average | 200 gms | 64.25mgms | 0.04181476 mgms | 0.0611% |

Quantity of adrenaline in suprarenals of the vitamin B-fed animals, by Folin, Cannon and Denis method.

| No | Wt. of animals | wt. of gland | Total adrenaline | Percentage |
|---|---|---|---|---|
| 3 | 220 gms | 49mgs | 0.0278516mgs | 0.05684% |
| 1. | 180 " | 47" | 0.030597 " | 0.0651 % |
| 2. | 100 " | 31 " | 0.023715 " | 0.0732% |
| 4. | 250 " | 52 " | 0.031638 " | 0.0608% |
| Average | 187.5 gms | 44.75mgms | 0.0284504 mgms | 0.05898% |

Quantity of adrenaline in suprarenals of the Vitamin B Deficient animals.

| No | Wt. of animals | Wt. of gland | Total adrenaline | percentage |
|---|---|---|---|---|
| 5. | 140 gms | 36mgms | 0.017136mgs | 0.047% |
| 6. | 140 " | 41 " | 0.020541 " | 0.0501% |
| 7. | 150 " | 47 " | 0.024612 " | 0.0528% |
| 8. | 185 " | 49 " | 0.025143 " | 0.0511 % |
| Average | 148.75"gms | 43.25mgms | 0.021858 mgms | 0.05001 % |

From these tables we find that the average ~~amount~~ percentage of adrenaline in the glands of animals on vitamin A containing diet was 0.043975% whereas that in the deficient animals it was 0.06111 % showing an increase of0.017125 % in the deficient animals.

Adrenaline in the glands of vitamin B deficiency animals on the average was 0.05001 % ,whereas in the controls the average was 0.05898% so that in the deficient animals there was a loss of 0.00897 %.

Blood pressure tracings of cats by injection of extracts of suprarenal glands of vitamin A and B fed and deficient animals roughly tally with the histological and chemical estimation of adrenaline in the glands.

Moreover the glands of animals that developed polyneuritis due to vitamin B deficiency (in extreme) showed a considerable amount of haemorrhage and distortion of the cells in the medulla as a result of acute insufficiency.

## Part II.

### Effects of vitamin C on thyroid and Suprarenals.

Fresh fruits and green vegetables which contain vitamin C in plenty are known to constitute an important part of any diet. In the present paper the effect of presence or absence of the antiscorbutic factor as it affects the structure of the thyroid and suprarenal glands is considered.

The animals experimented on, were rats, in batches of eight.One of these batches was fed with a diet deficient in the antiscorbutic vitamin; another batch which received raw cabbage and tomato in addition to the other constituents of the basal diet,served as control. The basal diet of both the batches was a mixture of Indian corn , wheat gluten, calcium carbonate, sodium chloride and milk, all boiled together. The difference in the diets of the two batches lay only in the presence or absence of vitamin C : in one there was plenty whereas in the other the vitamin was destroyed by prolonged boiling for at least an hour.

The animals were kept on these diets for two months. Each animal was weighed every week but neither of the two batches showed any appreciable gain or loss in their average weight during the whole of the experimental period. And clinical evidence of the development of scorbutic condition in the animals on vitamin C-deficient diet

diet was conspicuous by its absence. Rats seem to be less prone to scurvy than other animals, such as guinea-pigs which develop the disease easily. This has been noted by other observers.

The metabolism of the animals was recorded twice every week. Batch no 1 (with vitamin C) showed an almost constant metabolic rate as measured by the amount of oxygen consumed per hour, which varied from 1265 to 1280 c.c. whereas batch no 2 on deficient diet exhibited a slow but gradual increase in the oxygen consumption, from 1350 to 1415 c.c.

## Effect on thyroid.

Thyroid glands of the deficient animals were smaller and weighed much less than those of the normal animals; their average weight being 16 mgms whereas that of the controls was 29 mgms. The suprarenal glands did not show much difference in size and weight; the average weight of suprarenals of the deficient stock being 42 mgms as against 47 mgms of the normal animals.

Histologically, the thyroid glands of the animals on the deficient diet showed indications of moderately active secretion, the alveoli containing a very small quantity of colloid material and thick alveolar epithelium. The glands seemed congested; there was a fairly large aggregation of red blood cells between the alveoli with haemorrhagic patches here and there. The glands of the vitamin C fed animals exhibited a comparatively resting condition, the epithelium being thin with a full

with a full load of colloid material in the alveoli and without congestion.

Hewer (1) gives the following five methods of differential staining of the thyroid gland to show different phases of activity:-

| Reagents | I. Alkaline | II. Acid. |
|---|---|---|
| 1. Haematoxylin & Biebrich S. | Pink | Orange. |
| 2. Weigert's Resorcin-fuschin | Pale purple | Magenta. |
| 3. Aniline Blue -Orange G. | Blue | Orange. |
| 4. Haematoxylin and Congo Red. | Pink | Blue. |
| 5. Haematoxylin and Benzopurin | Yellow pink | Blue. |

According to her, freshly secreted colloid gives the alkaline reaction whereas the inactive or old material the acid.

Of these five methods I have tried two, viz, (a). Haematoxylin-Congo Red and (b). Aniline Blue-Orange G. Both showed the glands of the animals on vitamin C deficiency diet in a condition of greater secretory activity

activity than those of the controls. The haematoxylin-Congo red method showed, in the glands of the deficient animals, many alveoli containing pink red colloid material and the aniline blue- orange G method only had some alveoli blue( which according to Hewer indicates active secretion); while the control glands showed blue colloid in almost every alveolus by the haematoxylin-Congo red method and by aniline blue- orange G method numerous alveoli full of orange coloured colloid indicating according to Hewer, a resting condition of the glands.

Intravitam staining with neutral red showed no appreciable difference in the number of phagocytic cells in the glands of the deficiently fed animals as compared with those of the controls.

## The Acetonitril Test.

Reid Hunt's acetonitril test (2). for estimation of thyroid activity was tried on mice after feeding for six weeks with vitamin C and with vitamin C deficient diets. The animals averaged 20-25 gms in weight. Injection of acetonitril was commenced with a half minim dose dissolved in $\frac{1}{2}$ c.c. distilled water. Two of the three animals of the deficiency stock died after the first injection but the third survived the dose and succumbed to 3/4 m. A half minim of the same poison proved fatal to two of the normally fed animals but one of them required one minim

one minim dose to produce death. Judging from these injection experiments vitamin C is not antitoxic to acetonitril, in this respect resembling the other water-soluble vitamin B, whereas the fat-soluble A is definitely antitoxic to the drug.

A slow rise in the metabolic rate and histological sections of the gland prepared by different methods of staining indicate that deficiency of vitamin C (as with deficiency of vitamin A or B) leads to increased secretion on the part of the thyroid but does not cause a loss of weight.

## Effect on Suprarenals.

That normal diet containing vitamin C leads to an increase in the weight of the suprarenal glands, 47 mgms as against 42 mgms with diet deficient in that vitamin, has already been seen. The following table further shows that deficiency in the antiscorbutic factor leads to a diminution in the amount and percentage of adrenaline in the suprarenals as compared with those of the controls, although the difference was not as great as was found by Mc.Carrison (3) in his experiments on guineapigs. The fact that guineapigs are purely herbivorous and are accustomed to receive a large amount of vitamin C, probably renders them more susceptible to deficiency of that vitamin than other rodents such as rats which are omnivorous and whose normal diet contains less of the vitamin. This may be

may be why experimental scurvy is rare in rats. The difference in the amount of adrenaline is also less in them than in guineapigs. The results are given in the following tables.

Tables.

ADRENALINE CONTENT OF THE SUPRARENALS OF THE VITAMIN C FED ANIMALS.-Estimated by Folin, Cannon and Denis Method.(4)

| No of animals | weight | weight of glands | Total adrena -line | percentage |
|---|---|---|---|---|
| 6. | 125 gms | 46 mgms | 0.0289892mgms | 0.0692% |
| 2. | 245 " | 41 " | 0.0209100 " | 0.05186% |
| 13. | 205 " | 43 " | 0.0254800 " | 0.0582% |
| 5. | 240 " | 46 " | 0.0265880 " | 0.0578 % |
| Average | 205 Gms | 44mgms | 0.025497 mgms | 0.05819% |

ADRENALINE CONTENT OF THE SUPRARENALS OF THE VITAMIN C-DEFICIENT ANIMALS.

| No of Animals | weight | Weight of glands | Total adrenaline | percentage |
|---|---|---|---|---|
| 12. | 180 gms | 46 mgms | 0.03082 mgms | 0.067 % |
| 7. | 175 " | 46 " | 0.023276 " | 0.0506% |
| 9. | 170 " | 41 " | 0.01681 " | 0.0410% |
| 15. | 179 " | 42 " | 0.02372 " | 0.05287% |
| Average | 179 " | 43.75mgms | 0.02358 mgms | 0.05286% |

Histologically the glands, when stained by Cramer's osmicacid vapour method (5), corroborate the chemical estimation of the amount of adrenaline in the two series. The sections after osmic acid vapour staining(subsequently treated by turpentine oil to wash away the lipoid material) show that the adrenaline in the glands of the normal animals is in larger granules than that of the vitamin C deficient animals, in which it is in finer particles and in less quantity, while the lipoid content in the two series of glands does not show any appreciable difference.

A kymographic tracing of the blood pressure with injection of adrenaline extract of the glands into cats has provided another proof of the larger adrenaline content of the glands of normally fed animals as compared with that of the vitamin C- deficient animals.

## Part III.

## Effects of vitamin D on thyroid and Suprarenals.

According to K.H.Coward (1) the antirachitic activity of cod liver oil per g. ranges from 50 to 150 units showing a variation of 300%, whereas the milk in August contains only 0.2 unit per gram,which becomes reduced to a trace at the later part of the same month and disappears completely in the month of November. This antirachitic factor, which is missing in the October or November milk may be produced if the milk is subjected to ultra violet radiation for some time (2). Or the missing factor may be supplied to an animal taking the same milk by exposing the same animal to ultra violet rays occasionally.

Inorder to compare the effects of the different agents containing or capable of generating the antirachitic substances with those of diets deficient in vitamin D, the experimental animals (rats) were divided into three batches containing eight each. The basal diet of all these animals was a mixture of Indian corn, wheat gluten, biscuits, calcium carbonate and sodium chloride. Batch no 1 got in addition eight ounces of irradiated milk daily, as a supply of vitamin D. Batch No 2 got ordinary milk eight ounces every day but the animals were subjected to ultra violet radiation from a mercury vapour lamp from a distance of five feet for ten minutes twice every week. Batch no 3 which formed the deficiency stock

stock, got the same quantity of ordinary milk only, in addition to the basal diet. A fourth batch containing four animals only, received the same diet as batch no 3 but with the addition of a tea spoonful of cod liver oil daily. This batch was kept in a separate cage for better comparison with batches nos 1 and 2.

If we consider the opinion of Holmes, Wyman, Smith and Piggott (3). that 5 mg of cod liver oil as a protection against rickets is more effective than fifteen minutes irradiation at 36 inches, one can very well tabulate the quantity of antirachitic substance in the diet of the four batches of experimental animals thus:-

| Batch | Quantity of antirachitic substance received. |
|---|---|
| I | ++ (Irradiated milk). |
| II | + (Milk & ultra violet radiation) |
| III | — (milk only). |
| IV. | +++ (milk & cod liver oil). |

These animals were kept on the experimental diets for two months. The animals were very young, only two months old when the feeding was begun. All exhibited a normal growth. The average weight of the animals of batch I rose from 52 gms to 126 gms, of batch II from 48 gms to 128 gms, of batch III 54 to 127 gms whereas that of batch

that of batch IV rose from 67 to 142 gms. The fourth batch showed a slight gain in weight; the other three batches ran almost parallel with one another.

The metabolism of the animals was noted twice a week. Batch I consumed 700 c.c of oxygen per hour in the beginning as compared with 680 c.c. used by batch II and 700 c.c. by batch III. As all these animals were growing, their metabolism also increased gradually and steadily, sothat finally, when batch I could consume 1010 c.c. of oxygen per hour, batch II could take 1050 c.c. and batch III 1080 c.c. This showed a gradual increase in the metabolic rate from batch I to batch III.

## Effect on thyroid.

The average weight of thyroid glands of animals of batch I was 17 mgms; that of batch II, 21 mgms; that of batch III, 19 mgms; whereas that of batch IV was 16 mgms.

Histologically, by ordinary haematoxylin and eosin staining the glands of animals of batch IV showed a healthy normal structure, with alveoli almost all full of colloid, indicative of a full resting condition. Those of batch I showed a similar structure but a few alveoli were devoid of colloid material, indicating secreting activity on the part of these. Those of batch II showed

showed some alveoli full of colloid material but some had none especially towards the centre : the secreting epithelium was also thicker than in the other two batches. The glands of batch III showed a very few alveoli full of colloid material but many in a state of great secretory activity as shown by the absence of colloid material and the thick alveolar epithelium.

The Champy-Kull method (4) of staining corroborated these results beautifully and vividly.

By Hewer's (5) haematoxylin and Congo red method freshly secreted colloid substance taking a pink stain can be distinguished from inactive or old material taking a bluish stain : a similar differentiation is shown by her second method of staining by aniline blue and Orange G, by which freshly secreted colloid material taking a blue stain can be easily distinguished from old storage material staining orange.

The glands of the animals of batch IV showed a colloid material in all the alveoli stained bluish,by the Haematoxylin and Congo red method. By the same method of staining the glands of batch I exhibited all the alveoli full of bluish colloid material, with the exception of one or two showing a pink stain. The glands of batch II showed some of the alveoli full of pink colloid substance and some wuth blue material; whereas those of batch III exhibited almost all the alveoli full of pink material with the exception of a few containing blue substance.

By the aniline blue and orange G method, sections of glands of batch IV showed all the alveoli full of orange coloured colloid; those of batch I the same with the exception of a few containing blue material; those of batch II, some with blue and some with orange coloured colloid; those of batch III had almost all the alveoli full of colloid with the exception of a few here and there with orange coloured material.

By these different methods of staining, it could be shown that the glands of the animals of batch IV were in a full resting condition, those of batch I, nearly so ; those of batch II, intermediate between the resting and actively secreting phase; while the most actively secreting were those of batch III. The record of metabolism as previously related corroborates these results.

The therapeutic value of radiant energy of short wave lengths in the form of deep X-ray or radium in the treatment of excessive secretion of thyroid in exophthalmic and toxic goitre is well known. Cramer (6) has shown that physical agents such as heat also induce a resting condition in the thyroid.

Ultra violet rays, coming next to visible rays of the sun, occupy an intermediate position between heat and radiant energy in the electromagnetic field (7) as the following table illustrates:-

| Electric light | B.B.C wire-less | Infra-red (heat) | solar | ultra violet | x-ray | $\gamma$-ray radium |
|---|---|---|---|---|---|---|

The wave length of infra-red rays ranges from $3\times10^{-2}$ to $7\times10^{-5}$, that of the visible solar rays, from $7.2\times10^{-5}$ to $4\times10^{-5}$, that of the ultra violet rays from $3.5\times10^{-5}$ to $1\times10^{-6}$; that of X ray from $1\times10^{-7}$ to $2\times10^{-10}$; whereas that of radium rays is about $4\times10^{-12}$.

It is therefore quite possible that ultra violet radiation having a wave length of 300$\mu\mu$or less, while it produces the antirachitic subtance in the body, will be able partly to check the active secretion of the thyroid gland and afford it rest to some extent. The histological evidence is in accordance with this.

Vital staining with neutral red did not show any appreciable difference in the number of the phagocytic cells, in glands of the vitamin deficient animals,as compared with those of the batches getting vitamin D, whether in the form of cod liver oil irradiated milk or ultra violet radiation.

The Reid Hunt - reaction.(8).

Nine mice were taken in three batches and were fed on the same diet as batches I,II,and III of the rats and otherwise similarly treated. The average weight of the micewas nearly 30 gms. After two months subcutaneous injection of acetonitril was begun with a half minim dose dissolved in 0.5 c.c distilled water. All the animals (with the exception of one of batch I ) survived the dose.

A week later 3/4 m of the same drug dissolved in 0.5 c.c distilled water was injected into each. Unfortunately none of the animals were able to stand this dose: and all died. This shows that, vitamin D, although a fat soluble factor and closely associated with vitamin A, differs materially from vitamin A in not possessing the important antitoxic property.

## Effect on Suprarenals.

The average weight of the suprarenals of batch IV was 47 mgms; that of batch I,45 mgms; that of batch II, 40 mgms; while that of batch III was 41 mgms.

The following tables show the average total quantity and at the same time the percentage of adrenaline present in the glands of the three batches (a) on vitamin D (irradiated milk ) containing diet, (b) on vitamin D deficiency diet but exposed to ultra violet radiation and (c) on diet deficient in vitamin D, alone. The average total quantity in the glands of batch I was ( as estimated by the Folin, Cannon and Denis method ) 0.028388 mgms, that in batch II, 0.018672 mgms, whereas that in batch III was 0.022696 mgms. The percentages of adrenaline with respect to the whole gland were 0.0635 % , 0.04923 %,0.0594 %. in the animals of batches I,II, and III respectively.

The tables are given in the next page :-

Adrenaline content of the suprarenals of the vitamin D (irradiated milk )- fed animals estimated by Folin, Cannon and Denis Method.(9 ).

| No of Animals | Weight | Wt. of glands | total adrenaline | percentage. |
|---|---|---|---|---|
| 6. | 110 gms | 38mgms | 0.023028 mgms | 0.0606 % |
| 2. | 120 " | 40 " | 0.029340 " | 0.0718% |
| 1. | 130 " | 44 " | 0.028461 " | 0.0605 % |
| 4. | 170 " | 54 " | 0.032724 " | 0.0606 % |
| Average | 132.5gms | 44mgms | 0.028388 mgms | 0.0635 % |

Adrenaline content of the suprarenals of the vitamin D deficient animals exposed to ultra violet rays.

| No.of Animals | weight | wt of glands | total adrenaline | percentage. |
|---|---|---|---|---|
| 7. | 115gms | 48mgms | 0.026044 mgms | 0.05418 % |
| 12. | 110" | 30 " | 0.015990 " | 0.05332% |
| 10. | 140 " | 29 " | 0. 015043 " | 0.05182 % |
| 9. | 120 " | 37" | 0.017612 " | 0.04760 % |
| Average | 121gms | 36mgms | 0.018672 mgms | 0.04923 % |

Adrenaline content of the suprarenals of the animals with diet deficient in vitamin D.

| No.of animals | weight | wt of glands | Total adrenaline | percentage |
|---|---|---|---|---|
| 17. | 120gms | 38mgms | 0.026496 mgms | 0.0691 % |
| 15. | 85 " | 45 " | 0.023849 " | 0.0530 % |
| 16. | 140 " | 37 " | 0.019648 " | 0.0532 % |
| 14. | 140 " | 33 " | 0.020791 " | 0.0627 % |
| Average | 121gms | 38mgms | 0.022696 mgms | 0.0594 % |

Histologically the glands, when stained by Cramer's osmic acid vapour method (10) lead support to the quantitative chemical results. The cells of the medulla of the suprarenals of both batches I and IV exhibited the appearance of moderately active secretion, as shown by their slighly swollen and partially vacuolated appearance of the cells in the medullary portion, with a fairly large quantity of adrenaline in the form of black globules and granules, especially well seen in the clear cells. The medullary cells of batch III (those on deficiency diet), showed a slightly less quantity of adrenaline. The glands of batch II, on the other hand, showed an absolutely resting condition of the medullary cells,

medullary cells, with a few adrenaline granules scattered here and there. By ordinary haematoxylin and eosin staining, the resting condition of the medullary cells of the glands of batch II could be easily distinguished from the moderately actively secreting medullary cells in the glands of the animals of batches I, III, or IV.

Kymographic tracings of blood pressure wuth injection of the adrenaline extract of the glands into cats, also corroborate the histological and chemical results as regards the adrenaline content of the glands of the different batches of animals.

The lipoid content in the cortical portion of the glands does not show any appreciable difference in any of the four batches.

From these observations it is evident that vitamin D deficiency has only a slight effect on the suprarenals so far as the secretion of adrenaline is concerned. It leads to a slight diminution in the internal secretion of the medullary cells in comparison with that of control animals(batches I and IV). The resting condition of the medullary cells as well as the scanty amount of adrenaline present in the glands of the animals of batch II, is perhaps not due to presence or absence of the vitamin content but the direct effects of the ultra violet rays. Parhon and Orenstein (11) found that when non-albino animals are subjected to the influence of ultra violet rays, they develop richly pigmented cells. Those persons who expose themselves much to the sun as in the tropic, also develop

also develop similar pigmentation in the skin. Bloch(12) has shown that this pigment of the skin(melanin) is derived from a colourless predecessor closely related to dihydroxyphenylalanin which is a pyrocatechol derivative. As adrenaline is also a pyrocatechol derivative it is quite possible that when the medulla of the suprarenals are not functioning properly as in Addison's disease, the pyrocatechol derivative which would have formed adrenaline might be oxidised to melanin. Most probably such a factor comes into play when animals are subjected to ultra violet radiation occasionally, so that as a result of hypo-function of the medulla of the supra renals,more pigment is formedby the cells of the skin.

## C O N C L U S I O N.

### Part I .

1. Vitamin A containing diet affects the thyroid similarly to thyroid feeding in small doses, causing inactivity whereas deficiency leads to increased activity on the part of the gland.
2. A resting gland can exert its detoxicating function more than an actively secreting one. This is why vitamin A-fed animals can resist toxins better than the deficient ones.
3. Vitamin A acts specifically against Acetonitril like thyroid extract, as is shown by an increased number of phagocytic cells in the gland. The gland is kept in a resting phase so that it can counteract toxins. But when the glands become exhausted by active secretion under the influence of toxin, death takes place.
4. The growth promoting property of the vitamin A may be explained by diminished metabolism caused by producing the resting phase of the gland.
5. The growth promoting nature of vitamin B may be also explained by the diminished metabolism it causes.
6. The deficiency of vitamin B causes more increased secretion and ultimately hyperplasia of the thyroid.
7. Vitamin B is not antitoxic.
8. There is a striking similarity between the effects of

effects of vitamins A and B upon the thyroid gland (with the exception of the specific action of the vitamin A in resisting toxins).

9. Vitamin A or B deficiency alone cannot cause a complete absence of lipoid content of the cortex of the suprarenal gland.

10. Adrenaline content of the suprarenals is increased by vitamin A deficiency, whereas vitamin B deficiency produces a smaller amount than normal.

11. Vitamin B deficiency ultimately leads to an acute insufficiency of the medulla of the suprarenals.

## Part II.

1. Deficiency of vitamin C leads to an increase in the amount of secretion by the thyroid. Thus the antiscorbutic factor has an action on the thyroid similar to but less in amount than vitamins A and B.

2. Vitamin C like the other water soluble factor B is not antitoxic to acetonitril.

3. Adrenaline content of the suprarenals is diminished by a deficiency of vitamin C: in this respect it resembles vitamin B.

4. Vitamin C deficiency does not lead to any appreciable change in the lipoid content of the suprarenal cortex.

## Part III.

1. Vitamin D behaves towards the thyroid exactly as vitamins A,B and C. It promotes a resting condition of the gland and in this respect, substances like cod liver oil and irradiated milk which contain the antirachitic factor, or ultra violet rays which produce it, contribute towards the resting phase, proportional to the amount of the antirachitic substance they contain or are capable of producing. Deficiency of vitamin D leads to an actively secreting condition of the gland.

2. Vitamin D, although it is a fat soluble factor and is almost always closely associated with vitamin A, is not antitoxic to acetonitril.

3. Deficiency of vitamin D leads to a slight diminution in the adrenaline content of the suprarenal glands.

4. Ultra violet radiation leads to a scanty amount of adrenaline in the medulla of the suprarenals. This effect is probably due to the direct effect of the rays rather than to the chemical substance vitamin D, which is produced under their influence.

5. Vitamin D deficiency alone does not lead to any appreciable difference in the lipoid content of the cortex of the suprarenal glands.

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